Reference: *Biol. Bull.* **194:** 161–169. (April, 1998)

# Ion Transport in the Freshwater Bivalve *Corbicula fluminea*

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**Abstract.** In freshwater bivalves such as the mussel *Corbicula fluminea,* uptake of chloride depends on the external concentration of the chloride ion. In *C. fluminea,* $Cl^-$ uptake displayed saturation kinetics both in animals acclimated to pondwater and in those subjected to salt depletion by storage in deionized water. The transport capacity ($J_{max}$) was 7.00 ± 0.51 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ and the transport affinity ($K_m$) was 0.21 ± 0.08 m*M* in animals acclimated to pondwater. Animals subjected to salt depletion had a higher rate of $Cl^-$ uptake than did animals acclimated to pondwater. After 4 weeks in deionized water, the longer the animals were salt-depleted, the higher their rate of $Cl^-$ uptake. $Na^+$ and $Cl^-$ transport were independent in pondwater-acclimated *C. fluminea.* For salt-depleted animals, $Cl^-$ transport was $Na^+$-independent, but $Na^+$ transport depended partially on external $Cl^-$. Serotonin stimulated $Cl^-$ and $Na^+$ transport in pondwater-acclimated animals by increasing influx while having little influence on efflux. Acetazolamide increased the $Cl^-$ and $Na^+$ efflux of salt-depleted animals. Both serotonin and acetazolamide elevated the net loss of titratable base.

## Introduction

Freshwater bivalves have the same osmoregulatory problems as do other freshwater animals. They must accumulate salts from the dilute environment to compensate for the loss of solutes by diffusion and excretion. *Corbicula fluminea* (Müller) belongs to the family Corbiculidae and is a relatively recent invader of fresh water (Keen and Casey, 1969). It has a different blood ionic profile than the unionid clams (Dietz, 1979; McCorkle and Dietz, 1980; Byrne *et al.,* 1989), a family that invaded fresh water millions of years earlier (Keen and Casey, 1969). $Na^+$ and $Cl^-$ are the most important ions in the hemolymph of *C. fluminea,* accounting for 80%–90% of the total hemolymph solutes, compared to only about 60%–65% in unionids.

The rate of ion uptake in freshwater bivalves depends on the external ion concentration. Transport processes exhibit saturation kinetics in the freshwater bivalves that have been studied (Dietz, 1978; Dietz and Branton, 1979; McCorkle and Dietz, 1980; Dietz and Byrne, 1990; Dietz and Hagar, 1990; Wilcox and Dietz, 1995). In unionid clams, the transport capacity for $Na^+$, $Cl^-$, or $K^+$ is 1–2 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$, with a transport affinity of about 0.1 to 0.2 m*M* (Dietz, 1978; Dietz and Branton, 1979; Dietz and Byrne, 1990; Dietz and Hagar, 1990).

Blood osmolality is higher in *C. fluminea* than in the unionids (Dietz, 1979; McCorkle and Dietz, 1980; Byrne *et al.,* 1989), and the rate of ion transport is faster. Studies of $Na^+$ transport have shown that the transport capacity of *C. fluminea* is about 13 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$, or about 10 times the rate found in the unionid *Toxolasma (Carunculina) texasensis* (Dietz, 1978; McCorkle and Dietz, 1980). Potassium transport capacity in *C. fluminea* is twice that of unionids (Dietz and Byrne, 1990). The hemolymph $Cl^-$ and $Na^+$ regulation of *C. fluminea* has been studied recently (Byrne and Dietz, 1997), but transport kinetics have not been reported.

The $Na^+$-independence of $Cl^-$ uptake has been shown in many invertebrate and vertebrate species (Krogh, 1939; Romeu *et al.,* 1969; Stobbart, 1971; Kerstetter and Kirschner, 1972; Alvarado *et al.,* 1975; Lee and Pritchard, 1985). In the freshwater mussels that have been studied, $Na^+$ and $Cl^-$ transport are independent and $Cl^-$ uptake is thought to be *via* a $Cl^-/HCO_3^-$ exchange pathway (Murphy and Dietz, 1976; Dietz, 1978, 1985; Dietz and Branton, 1979; Dietz and Findley, 1979; Scheide and Dietz, 1982; Henry and Saintsing, 1983).

Received 22 April 1997; accepted 22 September 1997.

In this study, we characterize the transport kinetics of $Cl^-$ and demonstrate that $Na^+$ transport is partially $Cl^-$-dependent in salt-depleted *C. fluminea*. Both salt depletion and serotonin stimulate $Cl^-$ influx in *C. fluminea*.

## Materials and Methods

### *Animals*

Specimens of *Corbicula fluminea* were collected, under permit, from the Tangipahoa River in Mississippi. The animals were acclimated to aerated artificial pondwater (PW) containing (in m*M*) 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, and 0.05 KCl at 22°–25°C for at least 1 week before use. Salt-depleted (SD) clams were obtained by storing the animals in deionized water for at least 14 days. The deionized water was changed daily for the first 2 weeks and every other day for the rest of the salt-depletion period (McCorkle and Dietz, 1980).

### *Ion analysis*

The animals were rinsed for about 30 min in deionized water and transferred to individual beakers with appropriate bathing medium containing either $^{36}Cl^-$ or $^{22}Na^+$. For the experiments in which the net flux of titratable base was measured, Tris (tris(hydroxymethyl)aminomethane) was added into the bathing medium at a final concentration of 0.1 m*M*, and the pH was adjusted to 7.3 with Tris-HCl for either PW or $Na^+$-free PW, and with Tris-$H_2SO_4$ for $Cl^-$-free solutions. Choline was used to replace $Na^+$ in $Na^+$-free bathing medium, and sulfate was used to replace $Cl^-$ in $Cl^-$-free bathing medium. An initial (time zero) bath sample was taken after the clams began siphoning (within 10 to 15 min), with the second sample taken 2 h after time zero. $Cl^-$ concentration in the bath samples was determined by electrometric titration, and the $Na^+$ concentration was assayed by flame photometry. To measure the net loss of titratable base ($J_n^B$), samples were sonicated to remove respiratory $CO_2$. The samples were titrated to pH 4.5 with standardized 5 m*M* HCl, and the difference between initial and final buffer capacity was used to calculate net base production (Dietz and Branton, 1979).

### *Ion transport*

Unidirectional influx ($J_i$) was calculated from the disappearance of isotope (specific activity for $^{22}Na^+$ or $^{36}Cl^-$ was 2000 to 3000 CPM/$\mu M$) from the bathing medium, as previously described (Dietz, 1978; Graves and Dietz, 1982). Radioactivity was assayed with a liquid scintillation counter using cocktail based on Triton X-114/xylene (Wiegman *et al.*, 1975). Net flux ($J_n$) was estimated from the changes in bath ion concentration and normalized to $g^{-1}$ dry weight $h^{-1}$ (Dietz, 1978). To minimize the back flux of isotope, the bath volume was small (30 ml) to allow rapid changes in the radioactivity of the bath with limited isotope accumulating in the body fluids (Dietz, 1978).

In the study of $Cl^-$ transport kinetics, the unidirectional $Cl^-$ influx ($J_i^{Cl}$) was determined by a modification of the conventional methods described above. Because of the lack of sensitivity of the $Cl^-$ titrator, the $Cl^-$ net flux cannot be accurately measured if the $Cl^-$ concentration is less than 0.2 m*M*. This problem can be overcome by using a modified procedure, in which $Cl^-$ influx is determined from the change in $^{36}Cl^-$ in the bath divided by $^{36}Cl^-$ specific activity and normalized to grams of dry tissue. The bath $Cl^-$ concentration was measured in the initial stock solution and dilutions were assumed to have the same specific activity. We measured $Cl^-$ fluxes over the concentration range of 0.13 m*M* to 12.00 m*M*. To compare the two methods for determining $Cl^-$ influx, we calculated fluxes for animals exposed to six different $Cl^-$ concentrations and found no significant differences between these two data sets ($P > 0.25$). The agreement between these two rather independent estimates of $Cl^-$ influx thus validates our methods.

### *Administration of drugs*

Serotonin (5-hydroxytryptamine, 5HT) was added directly to the bathing medium at a final concentration of 0.1 m*M*. The pH of the solution was adjusted to 7.3 with NaOH after the addition of 5HT. Serotonin has been shown to stimulate $Na^+$ and $Cl^-$ uptake in other freshwater bivalves (see Dietz *et al.*, 1982; Horohov *et al.*, 1992). Acetazolamide (N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-acetamide, AZ) has been shown to alter ion fluxes in bivalves (see Henry and Saintsing, 1983). AZ was dissolved in bathing medium, and the animals were immersed in the solution at a final AZ concentration of 0.5 m*M* for 12 h before the experiments. No AZ was added to bathing medium during the flux measurement.

### *Statistics*

Data were expressed as mean ± standard error. In the study of $Cl^-$ transport kinetics, a rectangular hyperbolic function was chosen to fit the data (Inplot), and the Student's *t*-test was employed to examine the differences between the parameters of the function. In the studies of $Cl^-$ and $Na^+$ flux relationships, we employed a two-level factorial experimental design. These data were analyzed with SAS using a general linear model or a linear regression model. Differences were considered to be significant if the corresponding *P* value was equal to or less than 0.05.

## Results

### *Chloride transport kinetics*

The rate of unidirectional $Cl^-$ influx was dependent on the external $Cl^-$ concentration for both pondwater-acclimated (PWA) and salt-depleted (SD) *C. fluminea* (Fig. 1). When exposed to a range of NaCl concentrations, both PWA and SD animals displayed saturation kinetics. The $Cl^-$ uptake rate in PWA *C. fluminea* can be described by a Michaelis-Menten rectangular hyperbolic function ($R > 0.95$) with the maximum $Cl^-$ influx being the transport capacity ($J_{max}$) and the $Cl^-$ concentration at half of the $J_{max}$ representing the transport affinity ($K_m$). The $J_{max}$ for PWA animals was 7.46 ± 0.94 μeq $g^{-1}$ dry tissue $h^{-1}$ and the $K_m$ was 0.22 ± 0.15 m*M*. The $Cl^-$ influx in animals subjected to 2 weeks of salt depletion overlapped with that of PWA animals, and there was no significant difference between the two groups ($P > 0.25$). The hemolymph $Cl^-$ and $Na^+$ concentrations in animals subjected to 2 weeks of salt depletion were 20.2 ± 0.20 m*M* and 26.2 ± 0.31 m*M*, respectively. There was no statistical difference in the $Cl^-$ and $Na^+$ concentrations from PWA *C. fluminea* stored in the lab for the same period of time (20.9 ± 0.48 m*M* and 26.5 ± 0.31 m*M* for $Cl^-$ and $Na^+$, respectively, $n$ = 25–30, $P > 0.1$). Therefore, both data sets were combined to represent the $Cl^-$ uptake kinetics for PWA animals. This combined transport capacity and the corresponding transport affinity were calculated to be 7.00 ± 0.51 μeq $g^{-1}$ dry tissue $h^{-1}$ and 0.21 ± 0.08 m*M*, respectively.

Salt depletion of 4 weeks or longer resulted in the elevation of unidirectional $Cl^-$ uptake and a significant ($P < 0.01$) reduction in hemolymph $Cl^-$ and $Na^+$ concentrations (17.1 ± 0.23 m*M* and 22.5 ± 0.19 m*M* for $Cl^-$ and $Na^+$ after 4 weeks of SD, respectively, $n$ = 25–30). The $Cl^-$ influx of the animals subjected to salt depletion for 4 weeks or longer was consistently higher than that of the PWA animals for all $Cl^-$ concentrations tested. Compared to PWA animals, 4-week SD animals had an estimated increase in maximal $Cl^-$ influx ($J_{max}$ = 10.85 ± 0.91 μeq $g^{-1}$ dry tissue $h^{-1}$) of about 50%, and 8-week SD animals had about an 85% increase ($J_{max}$ = 13.49 ± 0.16 μeq $g^{-1}$ dry tissue $h^{-1}$). Animals subjected to 8 weeks of salt depletion exhibited higher net $Cl^-$ uptake rates than those found in animals salt-depleted for 4 weeks. Although the increase of the transport capacity in SD animals was substantial, the transport affinity for $Cl^-$ was not changed significantly.

### *The relationship between $Cl^-$ and $Na^+$ transport and the effects of 5HT on PWA animals*

Chloride transport was not affected by the absence of external $Na^+$ but was significantly stimulated by 5HT in PWA *C. fluminea* (Table I). With 5HT in the bath, animals displayed active siphoning, valve gaping, and extension of the foot as noted in other bivalves (Scheide and Dietz, 1984). Serotonin significantly increased influx and net flux of $Cl^-$ with little change in efflux compared to control *Corbicula*. $Cl^-$ net flux increased about 3.2 and 3.1 μeq $g^{-1}$ dry tissue $h^{-1}$ and influx about 2.0 and 2.3 μeq $g^{-1}$ dry tissue $h^{-1}$ in PW and $Na^+$-free PW bathing medium, respectively. No statistical interactions were found between external $Na^+$ and 5HT ($Na^+$/5HT), suggesting that the effect of 5HT on $Cl^-$ transport was independent of $Na^+$.

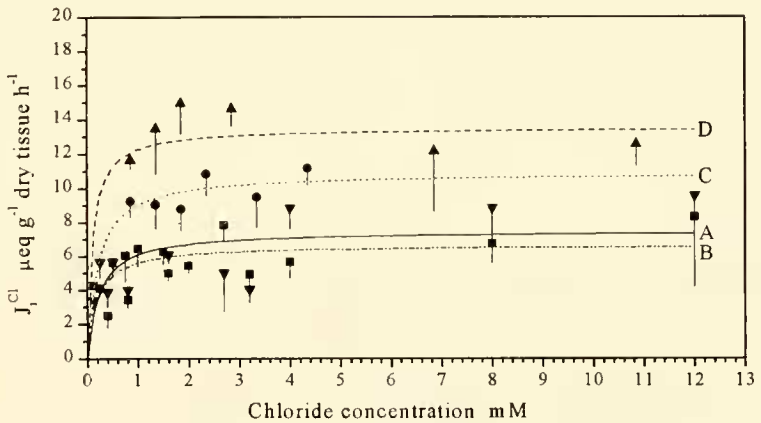


**Figure 1.** Effects of $Cl^-$ concentration on the average unidirectional $Cl^-$ influx in pondwater-acclimated (PWA) and salt-depleted (SD) *Corbicula fluminea*. Each point represents the mean and standard error of measurements for 5 animals. Down triangles (A): PWA animals, $J_{max}$ = 7.46 ± 0.94 μeq $g^{-1}$ dry tissue $h^{-1}$, $K_m$ = 0.22 ± 0.15 m*M*; Squares (B): SD for 2 weeks, $J_{max}$ = 6.63 ± 0.59 μeq $g^{-1}$ dry tissue $h^{-1}$, $K_m$ = 0.18 ± 0.10 m*M*; Circles (C): SD for 4 weeks, $J_{max}$ = 10.85 ± 0.91 μeq $g^{-1}$ dry tissue $h^{-1}$, $K_m$ = 0.20 ± 0.17 m*M*; Up triangles (D): SD for 8 weeks, $J_{max}$ = 13.49 ± 1.06 μeq $g^{-1}$ dry tissue $h^{-1}$, $K_m$ = 0.10 ± 0.12 m*M*.

**Table I**

*The effects of serotonin (5HT) and external $Na^+$ on $Cl^-$ transport and net flux of titratable base in pondwater-acclimated* Corbicula fluminea; *5HT was added to the bathing solution at a final concentration of 0.1 m*M

| | $+Na^+$ | | $-Na^+$ | | *F*-test | | |
|---|---|---|---|---|---|---|---|
| Flux | −5HT | +5HT | −5HT | +5HT | $\pm Na^+$ | ±5HT | $Na^+$/5HT |
| $J_n^{Cl}$ | −1.70 ± 0.63 | 1.45 ± 0.59 | −2.44 ± 0.48 | 0.70 ± 0.93 | | ** | |
| $J_i^{Cl}$ | 2.07 ± 0.30 | 4.07 ± 0.35 | 1.91 ± 0.37 | 4.23 ± 0.55 | | ** | |
| $J_o^{Cl}$ | 3.77 ± 0.81 | 2.62 ± 0.82 | 4.35 ± 0.69 | 3.53 ± 0.87 | | | |
| $J_n^{B}$ | −1.78 ± 0.39 | −4.19 ± 0.46 | −2.51 ± 0.51 | −5.73 ± 0.52 | * | ** | |

Data are means and standard errors of measurements for 10 animals and are expressed as $\mu$eq $g^{-1}$ dry tissue $h^{-1}$. $J_n$, net flux; $J_i$, influx; $J_o$, efflux; superscripts denote flux as either for chloride (Cl) or for titratable base (B). *: $P < 0.05$, **: $P < 0.01$. $Na^+$/5HT: interaction between $Na^+$ and 5HT.

The effect of 5HT on $Na^+$ transport was similar to that of $Cl^-$ transport (Table II). Serotonin increased $Na^+$ net flux 2.7 and 2.4 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$, and influx 2.3 and 1.6 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in PW and $Cl^-$-free PW, respectively. $Na^+$ transport was not affected by the absence of external $Cl^-$. There were no statistical interactions between external $Cl^-$ and 5HT ($Cl^-$/5HT), indicating that the effect of 5HT on $Na^+$ transport was not dependent on external $Cl^-$.

The loss of titratable base was significantly elevated by 5HT, but was independent of the external $Na^+$ or $Cl^-$, as indicated by the lack of effect of interactions between external $Cl^-$ or $Na^+$ and 5HT (Tables II and III). Serotonin increased the net flux of base by about 1.5 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ and 1.6 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in the presence and absence of external $Cl^-$, respectively. The absence of $Na^+$ caused a significant increase in the net loss of base. Increased loss of base was consistently observed in the absence of external $Na^+$ regardless of treatment with 5HT (0.73 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ increase in control animals and 1.54 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in 5HT treated animals).

Although $Cl^-$ and $Na^+$ uptakes were not directly dependent upon each other in PWA *C. fluminea*, as indicated by Tables I and II, a regression of paired $Cl^-$ and $Na^+$ net fluxes revealed a significant linear relationship (Fig. 2), in which a higher $Cl^-$ net flux correlated with a higher $Na^+$ net flux ($R = 0.84$; $P < 0.01$).

### *The relationship between $Cl^-$ and $Na^+$ transport and the effects of AZ on SD animals*

To study the effects of AZ on $Cl^-$ and $Na^+$ transport, we used SD *C. fluminea* to increase the basal rate of $Cl^-$ and $Na^+$ uptake. Studies with PWA animals were variable, and it was common to observe no effect of AZ on ion transport (data not shown). As shown in Table III, AZ was an effective inhibitor of net $Cl^-$ transport in SD *C. fluminea*. AZ pre-treatment resulted in a significant reduction of $Cl^-$ net flux by increasing $Cl^-$ efflux. The increase of $Cl^-$ efflux induced by AZ was 3.1 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in the presence of $Na^+$ and 6.0 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in the absence of $Na^+$. AZ exerted greater effects on $Cl^-$ efflux when there was no $Na^+$ in the bathing medium, as indicated by the significant interaction effect between external $Na^+$ and AZ (Table III).

**Table II**

*The effects of serotonin (5HT) and external $Cl^-$ on $Na^+$ transport and net flux of titratable base in pondwater-acclimated* Corbicula fluminea; *5HT was added to the bathing solution at a final concentration of 0.1 m*M

| | $+Cl^-$ | | $-Cl^-$ | | *F*-test | | |
|---|---|---|---|---|---|---|---|
| Flux | −5HT | +5HT | −5HT | +5HT | $\pm Cl^-$ | ±5HT | $Cl^-$/5HT |
| $J_n^{Na}$ | 1.31 ± 0.53 | 4.05 ± 0.33 | 1.43 ± 0.69 | 3.79 ± 0.51 | | ** | |
| $J_i^{Na}$ | 5.88 ± 0.68 | 8.21 ± 0.29 | 5.95 ± 0.47 | 7.57 ± 0.86 | | ** | |
| $J_o^{Na}$ | 4.57 ± 0.34 | 4.16 ± 0.17 | 4.52 ± 0.30 | 3.78 ± 0.44 | | | |
| $J_n^{B}$ | −2.72 ± 0.51 | −4.18 ± 0.54 | −2.23 ± 0.55 | −3.82 ± 0.68 | | ** | |

Data are means and standard errors of measurements for 10 animals and are expressed as $\mu$eq $g^{-1}$ dry tissue $h^{-1}$. $J_n$, net flux; $J_i$, influx; $J_o$, efflux; superscripts denote flux as either for sodium (Na) or for titratable base (B). *: $P < 0.05$, **: $P < 0.01$. $Cl^-$/5HT: interaction between $Cl^-$ and 5HT.

**Table III**

*The effects of acetazolamide (AZ) and external $Na^+$ on $Cl^-$ transport and net flux of titratable base in salt-depleted* Corbicula fluminea; *the animals were pre-treated for 12 h before the experiments with AZ added to the bathing solution at a final concentration of 0.5 mM (No AZ was added to the bathing solution during the experiments)*

| | +$Na^+$ | | −$Na^+$ | | *F*-test | | |
|---|---|---|---|---|---|---|---|
| Flux | −AZ | +AZ | −AZ | +AZ | ±$Na^+$ | ±AZ | $Na^+$/AZ |
| $J_n^{Cl}$ | 3.89 ± 1.18 | 2.17 ± 0.88 | 4.46 ± 0.77 | 1.73 ± 0.59 | | * | |
| $J_i^{Cl}$ | 6.41 ± 1.07 | 7.77 ± 0.68 | 5.27 ± 0.57 | 8.55 ± 0.60 | | ** | |
| $J_o^{Cl}$ | 2.52 ± 0.62 | 5.60 ± 0.89 | 0.81 ± 0.51 | 6.82 ± 0.73 | | ** | * |
| $J_n^{B}$ | −6.95 ± 1.37 | −10.83 ± 1.08 | −5.65 ± 0.69 | −8.83 ± 0.48 | | ** | |

Data are means and standard errors of measurements for 10 animals and are expressed as $\mu$eq $g^{-1}$ dry tissue $h^{-1}$. $J_n$, net flux; $J_i$, influx; $J_o$, efflux; superscripts denote flux as either for chloride (Cl) or for titratable base (B). *: $P < 0.05$, **: $P < 0.01$. $Na^+$/AZ: interaction between $Na^+$ and AZ.

$Na^+$ efflux was also elevated by AZ—by about 1.8 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in the presence of external $Cl^-$ and 3.6 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in the absence of external $Cl^-$ (Table IV). Net flux remained unchanged owing to the small but significant increase in $Na^+$ influx by AZ treatment.

Unlike PWA *C. fluminea*, in which $Cl^-$ and $Na^+$ transport were independent as described above, the $Cl^-$ and $Na^+$ transport in SD animals appeared to be more interrelated (Table IV). Although $Cl^-$ uptake was $Na^+$-independent in SD *C. fluminea*, as indicated by the lack of alteration of $Cl^-$ influx by the absence of external $Na^+$, $Na^+$ uptake was significantly reduced by the absence of external $Cl^-$. With or without exposure to AZ, there was a 3 to 4 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ reduction in $Na^+$ influx in the absence of external $Cl^-$, resulting in the significant decrease in the $Na^+$ net flux. This suggests that the component of $Na^+$ uptake stimulated by salt depletion is at least partially dependent on the presence of external $Cl^-$.

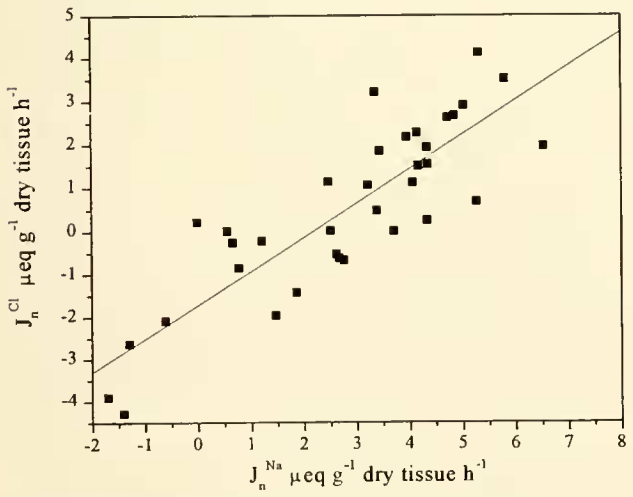


**Figure 2.** Relationship between net fluxes of $Cl^-$ and $Na^+$ in pondwater-acclimated *Corbicula fluminea*. $J_n$, net flux, expressed as $\mu$eq $g^{-1}$ dry tissue $h^{-1}$. $J_n^{Cl} = -1.73 + (0.79)\ J_n^{Na}$, $R = 0.85$, $n = 35$, $P < 0.01$.

As shown in Tables III and IV, the effect of AZ on base net flux was consistent in the two experiments with SD *C. fluminea*. AZ significantly increased the net loss of titratable base. The increase of net base flux was about 4 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in PW medium, 3.2 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in $Na^+$-free bathing medium, and 1.9 $\mu$eq $g^{-1}$ dry tissue $h^{-1}$ in $Cl^-$-free bathing medium. No statistical interaction between external $Cl^-$ or $Na^+$ and AZ was observed, indicating that the net flux of base was not affected by the absence of external $Cl^-$ or $Na^+$.

In both PWA and SD *C. fluminea*, the net flux of titratable base was not affected by the absence of external $Cl^-$. However, a regression between $Cl^-$ influx and net flux of base from individual animals demonstrated a significant linear relationship (Fig. 3, $R = 0.78$ and 0.82 for SD and PWA animals, respectively, $P < 0.01$). In all experiments, a greater $Cl^-$ influx correlated with a greater base net flux. The slope of the regression for PWA animals was not statistically different from that of SD animals, suggesting that the relationship between $Cl^-$ influx and base net flux is stoichiometrically similar in both acclimation conditions.

## Discussion

### *The relationship between $Cl^-$ and $Na^+$ transport in* C. fluminea

$Na^+$ transport in *C. fluminea* may be $Cl^-$-independent or $Cl^-$-dependent, depending on the acclimation conditions. $Na^+$ transport and the component of $Na^+$ active uptake stimulated by 5HT were $Cl^-$-independent in PWA (pondwater-acclimated) animals. However, a substantial fraction of $Na^+$ uptake in SD (salt-depleted) *C. fluminea* depended on the presence of external $Cl^-$. These data indicate that multiple pathways for $Na^+$ uptake may exist

**Table IV**

*The effects of acetazolamide (AZ) and external $Cl^-$ on $Na^+$ transport and net flux of titratable base in salt-depleted* Corbicula fluminea; *the animals were pre-treated for 12 h before the experiments with AZ added to the bathing solution at a final concentration of 0.5 mM (no AZ was added to the bathing solution during the experiments)*

| | $+Cl^-$ | | $-Cl^-$ | | *F*-test | | |
|---|---|---|---|---|---|---|---|
| Flux | −AZ | +AZ | −AZ | +AZ | ±Cl | ±AZ | Cl/AZ |
| $J_n^{Na}$ | 7.75 ± 0.92 | 7.18 ± 0.72 | 5.38 ± 0.90 | 3.81 ± 0.83 | * | | |
| $J_i^{Na}$ | 12.33 ± 1.34 | 13.55 ± 1.12 | 8.45 ± 1.64 | 10.49 ± 0.87 | * | | |
| $J_o^{Na}$ | 4.58 ± 0.50 | 6.37 ± 0.65 | 3.07 ± 0.79 | 6.69 ± 0.82 | | ** | |
| $J_n^B$ | −1.47 ± 0.97 | −5.74 ± 0.70 | −2.46 ± 1.02 | −4.33 ± 0.53 | | ** | |

Data are means and standard errors of measurements for 10 animals and are expressed as μeq $g^{-1}$ dry tissue $h^{-1}$. $J_n$, net flux; $J_i$, influx; $J_o$, efflux; superscripts denote flux as either for sodium (Na) or for titratable base (B). *: $P < 0.05$, **: $P < 0.01$. Cl/AZ: interaction between $Cl^-$ and AZ.

in *C. fluminea*, and that the pathway stimulated by salt depletion is different from that stimulated by 5HT. A $Na^+/H^+$ exchange mechanism has been considered to be a major pathway for $Na^+$ uptake in freshwater bivalves including *C. fluminea* (McCorkle and Dietz, 1980; Dietz, 1978, 1985; Henry and Saintsing, 1983; Byrne and Dietz, 1997). However, other $Na^+$ uptake pathways, such as indirect coupling to the activity of a $H^+$-translocating-ATPase, may also exist. Recent studies have demonstrated that $Na^+$ uptake in the gills of freshwater rainbow trout is energized by the electrogenic $H^+$-ATPase located on the apical membrane of the gill (Lin and Randall, 1995).

Serotonin stimulated the $Cl^-$ influx by increasing the active transport component, presumably a $Cl^-/HCO_3^-$ exchange pathway (see below). The lack of effect of external $Na^+$ on the stimulation of $Cl^-$ uptake by 5HT suggests that the active uptake of $Cl^-$ in *C. fluminea* is a $Na^+$-independent $Cl^-/HCO_3^-$ exchange. Salt-depletion was reported to stimulate the activity of carbonic anhydrase (CA) in the gills of *C. fluminea* (Henry and Saintsing, 1983). The increased amount of $HCO_3^-$ synthesized due to the CA activity may stimulate a $Cl^-/HCO_3^-$ exchange pathway directly or indirectly by providing more $HCO_3$ ion. Independent transport of $Cl^-$ and $Na^+$ is common in freshwater mussels (Dietz, 1978; Dietz and Branton, 1979; Dietz and Hagar, 1990). In this respect, PWA *C. fluminea* is similar to other freshwater bivalves studied.

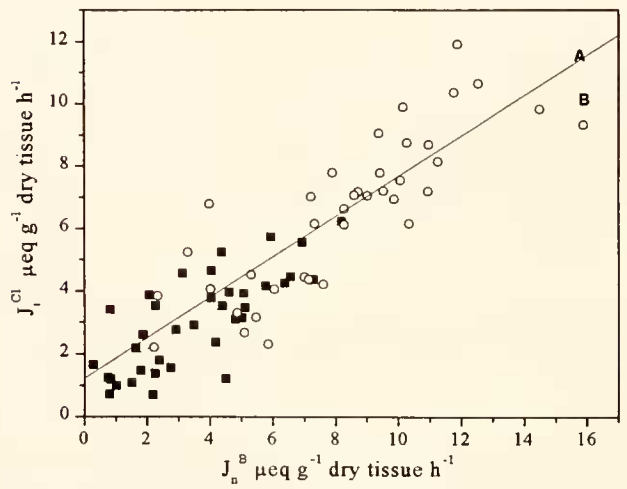


**Figure 3.** Relationship between $Cl^-$ influx and net loss of titratable base in pondwater-acclimated (PWA) and salt-depleted (SD) *Corbicula fluminea*. (A) Open circle, SD animals, $J_i^{Cl} = 1.04 + (0.56)\ J_n^B$, $R = 0.77$, $n = 37$, $P < 0.01$. (B) Solid square, PWA animals, $J_i^{Cl} = 1.98 + (0.65)\ J_n^B$, $R = 0.82$, $n = 38$, $P < 0.01$. $J_n$, net flux, expressed as μeq $g^{-1}$ dry tissue $h^{-1}$. The slopes of two lines (A = 0.65 ± 0.07; B = 0.56 ± 0.08) were not significantly different.

*The effect of 5HT on ion transport in* C. fluminea

The effects of 5HT on $Cl^-$ transport in this study are similar to the results reported by Byrne and Dietz (1997). Cyclic AMP and other tested agents had no apparent effect on $Cl^-$ net flux in *Toxolasma texasensis* and *Ligumia subrostrata* (Scheide and Dietz, 1986). Since the 5HT-stimulated $Cl^-$ uptake was independent of external $Na^+$, 5HT must exert its effect directly on $Cl^-$ uptake rather than indirectly by stimulating $Na^+$ uptake.

Chloride/bicarbonate exchange is an important mechanism for $Cl^-$ uptake in the freshwater bivalves that have been studied. Stimulation of ATPase activity by chloride and $HCO_3$ has been demonstrated in the microsomal membranes of the gills of unionid mussel *Toxolasma texasensis* (Dietz and Findley, 1979). The highly significant correlation between $Cl^-$ influx and the net flux of titratable base in both PWA and SD *C. fluminea* in this study also suggests the importance of this exchange mechanism in $Cl^-$ uptake. In PWA *C. fluminea*, the active uptake component *via* $Cl^-/HCO_3^-$ exchange and the exchange diffusion through $Cl^-/Cl^-$ turnover both potentially contributes to total $Cl^-$ isotope turnover. The exchange diffusion pathway was demonstrated to be important for the movement of $^{22}Na^+$ isotope in *C. fluminea* (McCorkle and

Dietz, 1980). However, exchange diffusion of $Cl^-$ was not significant in *C. fluminea*, because 5HT significantly increased $Cl^-$ influx and net flux with no change in $Cl^-$ efflux. In this study we observed a stoichiometric $Cl^-/HCO_3^-$ exchange (Fig. 3) and the small but consistent decrease of average net base flux in the absence of external $Cl^-$ (Table II).

The stimulation of net flux of titratable base by 5HT has not been reported previously in freshwater bivalves. In this study, the net flux of titratable base was measured by determining the amount of exogenous acid required to titrate the base in the bathing solution. However, the amount of exogenous acid required can be affected by the amount of endogenous proton released from the bivalve into the bathing solution. Therefore, the net change of titratable base reflects the combined results of the activities of $Cl^-/HCO_3^-$ exchanger, $Na^+/H^+$ antiporter, and $H^+$-ATPase in renal and extrarenal (gills) tissue. The stimulation of $Na^+$ uptake may extrude more protons, presumably through $Na^+/H^+$ antiporter or $H^+$-pump (or both), that would autotitrate the base in the bathing solution. The activation of $Cl^-$ uptake would elevate the amount of base in the medium *via* $Cl^-/HCO_3^-$ exchanger. In PWA *C. fluminea*, 5HT significantly increased the net flux of titratable base, and the increase was enhanced when $Na^+$ was not present in the bathing medium (Table I).

Although the increase of $Na^+$ and $Cl^-$ uptake by 5HT accounts for some fraction of the alteration of net base flux, a substantial proportion of the net base flux that was stimulated by 5HT was independent of the uptake of $Na^+$ and $Cl^-$. This was indicated by the observation that in the absence of external $Na^+$ or $Cl^-$ there was a persistent loss of base that was elevated by 5HT (Tables I and II). The mechanism for the elevation of base loss by 5HT is not clear. It could be an indirect result of the increased amount of $HCO_3^-$ filtered by the renal tissue (Dietz and Byrne, 1997). 5HT appears to be a "universal" stimulator of freshwater bivalves including *C. fluminea*. Its effects on freshwater mussels include increases in gill ciliary activity, foot movement, and valve closure; gill muscle relaxation; and induction of spawning and other reproductive activities (Scheide and Dietz, 1984; Gardiner *et al.*, 1991; Fong *et al.*, 1993, 1994). The stimulation of all these activities may increase metabolic $CO_2$ production and result in the elevation of blood $HCO_3^-$ due to the hydration of $CO_2$. Alternatively, 5HT may directly stimulate $HCO_3^-$ excretion in the renal tissue of *C. fluminea*.

### *The effects of AZ on ion transport in* C. fluminea

Whereas 5HT acts on the $Cl^-$ and $Na^+$ uptake process in the gills of SD *C. fluminea*, AZ works predominantly on the $Cl^-$ and $Na^+$ exit pathway. AZ depressed $Cl^-$ transport of SD *C. fluminea* by exerting a greater influence on the efflux of the ion than on its influx. The significant increase in $Cl^-$ efflux produced by AZ in this study is consistent with the results of previous studies of the freshwater mussels *Toxolasma texasensis* and *Ligumia subrostrata* (Dietz and Branton, 1979; Henry and Saintsing, 1983). An increase in $Cl^-$ influx, on the other hand, has not been previously reported as an effect of AZ. AZ has been shown to inhibit $Na^+$ transport by reducing the influx in *Ligumia subrostrata* (Henry and Saintsing, 1983). In SD *C. fluminea*, however, AZ exerted its effect exclusively on the $Na^+$ efflux, and no evidence of a reduction in influx was observed. This disagreement may be due to the species differences in response to AZ or in the $Na^+$ uptake process.

The effect of AZ on the flux of titratable base has not been reported previously in freshwater bivalves. In SD *C. fluminea*, AZ significantly increased the net loss of base. Although gill epithelia are an important site for the exit of $HCO_3^-$ and $H^+$ through the exchange activities, filtration and excretion by the renal tissue is also a significant process in the regulation of acid and base balance. An increase of blood $HCO_3^-$ by AZ treatment has been demonstrated in *C. fluminea* and other freshwater mussels (Henry and Saintsing, 1983). The blockage of $HCO_3^-$ exit pathways in the gill epithelia would result in the accumulation of $HCO_3^-$ in the blood (Byrne and Dietz, 1997) and increase the amount of $HCO_3^-$ being subjected to filtration and excretion by the renal tissue.

### *$Cl^-$ transport kinetics*

Unidirectional ion uptake depends on the corresponding external ion concentration and displays saturation kinetics in the freshwater bivalves studied (Dietz, 1978; Dietz and Branton, 1979; McCorkle and Dietz, 1980; Dietz and Byrne, 1990; Dietz and Hagar, 1990; Wilcox and Dietz, 1995). A high ion transport capacity ($J_{max}$) seems to be a common characteristic in *C. fluminea*. The $Cl^-$ transport capacity is about 7 times higher in *C. fluminea* than in the unionids *Toxolasma texasensis* and *Ligumia subrostrata* (Dietz and Branton, 1979; this study).

In this study, $Cl^-$ transport capacity was estimated to be increased 50% and 85% after salt depletion for 4 and 8 weeks, respectively. Two weeks of salt depletion is sufficient to stimulate the rate of ion uptake in most unionid clams, and even less time is required in zebra mussels (Dietz, 1985; Horohov *et al.*, 1992; Dietz *et al.*, 1994). For *C. fluminea*, however, a significant elevation of $Cl^-$ influx requires at least 4 weeks of salt depletion. The prolonged time requirement suggests that this species has a low epithelial permeability to ions (Zheng and Dietz, 1998). The transport affinity for $Cl^-$ that we measured in this study is about 0.2 m*M* for PWA and SD *C. fluminea*.

The elevation in ion transport capacity with little change in transport affinity suggests that more epithelial transport sites are activated during salt depletion.

Studies of transport kinetics using radioactive Rb as a substitute tracer for K in the zebra mussel, *Dreissena polymorpha,* have revealed a greater proportion of diffusive uptake at higher external Rb concentrations (Wilcox and Dietz, 1995). The ion concentrations used in transport kinetic studies in freshwater bivalves is usually under 3 m*M*. In this study, we applied $Cl^-$ concentrations up to 12 m*M* in an attempt to identify a passive diffusion component of $Cl^-$ uptake. Passive diffusion would appear as a linear increase in flux at higher ion concentrations (see Wilcox and Dietz, 1995). A Michaelis-Menten rectangular hyperbolic function fit the $Cl^-$ influx data for PWA *C. fluminea* very well ($R > 0.95$), with no evidence of a diffusive component. An attempt to study fluxes at higher $Cl^-$ concentrations (up to 20 m*M*) was unsuccessful because the animals did not open their valves.

The characteristics of $Cl^-$ uptake in *C. fluminea* reveal differences compared with those of other freshwater bivalves such as unionids and zebra mussels (Dietz and Branton, 1979; Dietz *et al.*, 1995; Byrne and Dietz, 1997; Zheng and Dietz, 1998). The epithelium of *C. fluminea* is much "tighter" than that of unionids or zebra mussels. The tight, or low, permeability of the epithelium in this species is reflected by the longer time required for salt depletion and the lack of evidence for a passive diffusion component in $Cl^-$ uptake. *C. fluminea* has a higher blood osmolality than do unionids and zebra mussels and has been reported to inhabit brackish water with salinities as high as 5‰ (Filice, 1958; Hayashi, 1956; Keen and Casey, 1969). In addition, this species is very tolerant of salt depletion and can be kept in deionized water for several months with little mortality (McCorkle and Dietz, 1980). Thus, *C. fluminea* can tolerate a much broader range of ambient osmolality than unionid clams and zebra mussels.

## Acknowledgments

We thank Chengsung Jiang, Janice E. Horohov, Julie Cherry, Diondi Lessard, Paul Bruce, Shawn Wilcox, and Timothy Smith for technical assistance. Harold Silverman provided valuable comments. The paper was submitted to the Graduate School of Louisiana State University and A&M College in partial fulfillment of the Ph. D degree by H. Z. The research was supported in part by NSF 90-17461.

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